(12) UK Patent Application (19) GB (11) 2 291 422 (13) A

(43) Date of A Publication 24.01.1996

(21) Application No 9414453.2

(22) Date of Filing 18.07.1994

(71) Applicant(s)
Fujisawa Pharmaceutical Co.,Ltd

(Incorporated in Japan)

4-7, Doshomachi 3-chome, Chuo-ku, Osaka-shi, Osaka 541, Japan

(72) Inventor(s)
Norihiko Shimazaki
Shinya Watanabe
Akihiko Sawada
Keiji Hemmi

(74) Agent and/or Address for Service
Stevens, Hewlett & Perkins
1 Serjeant's Inn, Fleet Street, LONDON, EC4Y 1LL, United Kingdom

(51) INT CL^6
C07D 471/04 // (C07D 471/04 213:00 241:00)

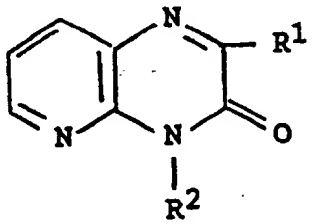
(52) UK CL (Edition O)
C2C CAA CKD CKM CKS CTR C155X C214 C22Y C220 C226 C25Y C250 C252 C281 C30Y C305 C34Y C342 C351 C352 C36Y C364 C366 C368 C386 C387 C601 C625 C626 C628 C65X C678 C699 C71X C761 C762 C80Y C802
U1S S1313 S2411 S2416

(56) Documents Cited
EP 0008864 A

(58) Field of Search
INT CL^6 C07D 471/04
Online databases: CAS-ONLINE, EDOC, JAPIO, WPI

(54) 4-phenyl-pyrido[2,3-b]pyrazin-4-ones

(57) Pyridopyrazine derivatives of the following formula:-



wherein R^1 is lower alkyl, carboxy or protected carboxy, and
R^2 is aryl which may have protected amino,
and pharmaceutically acceptable salts thereof.

A process for preparing such compounds is also described. These compounds possess inhibitory activity against both phosphodiesterase IV (PDE-IV) and the production of tumour necrosis factor (TNF), and can therefore be used for the prophylactic and therapeutic treatment of PDE-IV and TNF mediated diseases, such as chronic inflammatory diseases, autoimmune diseases and sepsis-induced organ injury.

GB 2 291 422 A

2291422

NEW HETEROBICYCLIC DERIVATIVES

This invention relates to new heterobicyclic derivatives. More particularly, this invention relates to pyridopyrazine derivatives and pharmaceutically acceptable salts thereof which have pharmacological activities, processes for preparation thereof, a pharmaceutical composition comprising the same and a use of the same.

Accordingly, one object of this invention is to provide the new and useful pyridopyrazine derivatives and pharmaceutically acceptable salts thereof which possess a strong phosphodiesterase IV (PDE IV)-inhibitory activity and a strong inhibitory activity on the production of tumor necrosis factor (TNF).

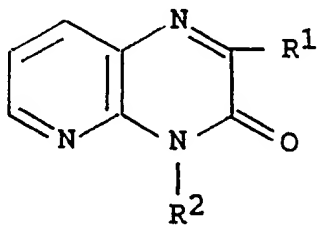
Another object of this invention is to provide processes for preparation of the pyridopyrazine derivatives and salts thereof.

A further object of this invention is to provide a pharmaceutical composition comprising said pyridopyrazine derivatives or a pharmaceutically acceptable salt thereof.

Still further object of this invention is to provide

a use of said pyridopyrazine derivatives or a pharmaceutically acceptable salt thereof as a medicament for prophylactic and therapeutic treatment of PDE-IV and TNF mediated diseases such as chronic inflammatory diseases, specific autoimmune diseases, sepsis-induced organ injury, and the like in human being and animals.

The object pyridopyrazine derivatives of the present invention are novel and can be represented by the following general formula (I) :



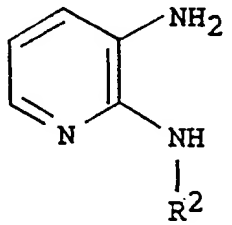
(I)

wherein R^1 is lower alkyl, carboxy or protected carboxy, and

R^2 is aryl which may have protected amino.

The object compound (I) of the present invention can be prepared by the following process.

Process (1)

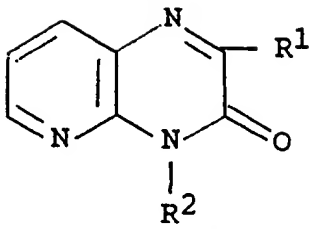


(II)

or a salt thereof

$$HOOC-\overset{\overset{O}{\|}}{C}-R^1$$

(III)
or a salt thereof

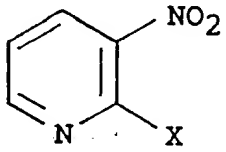


(I)
or a salt thereof

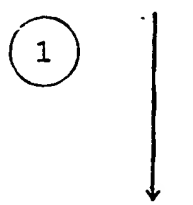
wherein R^1 and R^2 are each as defined above.

The starting compound (II) of the present invention can be prepared by the following processes.

Process (A)



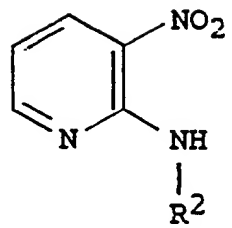
(IV)
or a salt thereof



(1)

$$H_2N-R^2$$

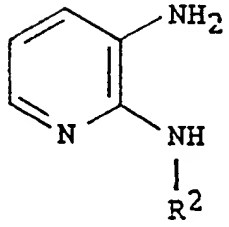
(V)
or a salt thereof



(VI)

or a salt thereof

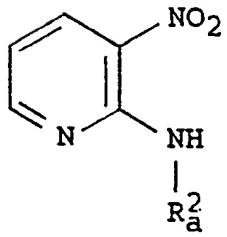
(2) reduction



(II)

or a salt thereof

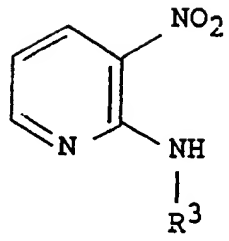
Process (B)



(VIa)

or a salt thereof

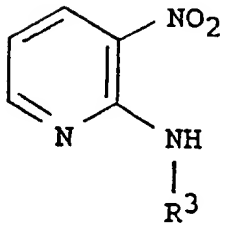
↓ elimination reaction of the amino protective group



(VII)

or a salt thereof

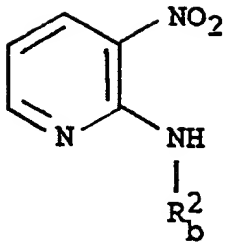
Process (C)



(VII)

or a salt thereof

↓ acylation



(VIb)

or a salt thereof

wherein R^2 is as defined above,
R^3 is aryl having amino,
R^2_a is aryl having protected amino,
R^2_b is aryl having acylamino, and
X is a leaving group.

Suitable pharmaceutically acceptable salts of the object compound (I) are conventional non-toxic salts and may include a salt with a base or an acid addition salt such as a salt with an inorganic base, for example, an alkali metal salt (e.g., sodium salt, potassium salt, etc.), an alkaline earth metal salt (e.g., calcium salt, magnesium salt, etc.), an ammonium salt; a salt with an organic base, for example, an organic amine salt (e.g., triethylamine salt, pyridine salt, picoline salt, ethanolamine salt, triethanolamine salt, dicyclohexylamine salt, N,N'-dibenzylethylenediamine salt, etc.); an inorganic acid addition salt (e.g., hydrochloride, hydrobromide, sulfate, phosphate, etc.); an organic carboxylic or sulfonic acid addition salt (e.g., formate, acetate, trifluoroacetate, maleate, tartrate, fumarate, methanesulfonate, benzenesulfonate, toluenesulfonate, etc.); a salt with a basic or acidic amino acid (e.g., arginine, aspartic acid, glutamic acid, etc.).

In the above and subsequent descriptions of the present specification, suitable examples and illustration

of the various definitions which the present invention intends to include within the scope thereof are explained in detail as follows.

The term "lower" is used to intend a group having 1 to 6, preferably 1 to 4, carbon atom(s), unless otherwise provided.

The term "higher" is used to intend a group having 7 to 20 carbon atoms, unless otherwise provided.

Suitable "lower alkyl" may include straight or branched one having 1 to 6 carbon atom(s), such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, tert-pentyl, hexyl, and the like.

Suitable "lower alkoxy" may include methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, t-butoxy, pentyloxy, t-pentyloxy, hexyloxy and the like.

Suitable "aryl" may include phenyl, naphthyl and the like.

Suitable "halogen" may include fluorine, bromine, chlorine and iodine.

Suitable "leaving group" may include acid residue, lower alkoxy as exemplified above, and the like.

Suitable "acid residue" may include halogen as exemplified above, acyloxy and the like.

Suitable "protected carboxy" may include esterified carboxy and the like. And suitable example of said ester may be the ones such as lower alkyl ester (e.g., methyl ester, ethyl ester, propyl ester, isopropyl ester, butyl ester, isobutyl ester, t-butyl ester, pentyl ester, t-pentyl ester, hexyl ester, etc.); lower alkenyl ester (e.g., vinyl ester, allyl ester, etc.); lower alkynyl ester (e.g., ethynyl ester, propynyl ester, etc.); lower alkoxy(lower)alkyl ester (e.g., methoxymethyl ester, ethoxymethyl ester, isopropoxymethyl ester, 1-methoxyethyl ester, 1-ethoxyethyl ester, etc.); lower alkylthio(lower)-alkyl ester (e.g., methylthiomethyl ester, ethylthiomethyl

ester, ethylthioethyl ester, isopropoxythiomethyl ester, etc.); mono(or di or tri)halo(lower)alkyl ester (e.g., 2-iodoethyl ester, 2,2,2-trichloroethyl ester, etc.); lower alkanoyloxy(lower)alkyl ester (e.g., acetoxymethyl ester, propionyloxymethyl ester, butyryloxymethyl ester, valeryloxymethyl ester, pivaloyloxymethyl ester, hexanoyloxymethyl ester, 1-acetoxyethyl ester, 2-acetoxyethyl ester, 2-propionyloxyethyl ester, etc.); lower alkoxycarbonyloxy(lower)alkyl ester (e.g., methoxycarbonyloxymethyl ester, ethoxycarbonyloxymethyl ester, propoxycarbonyloxymethyl ester, 1-(or 2-)-[methoxycarbonyloxy]ethyl ester, 1-(or 2-)-[ethoxycarbonyloxy]ethyl ester, 1-(or 2-)-[propoxycarbonyloxy]ethyl ester, 1-(or 2-)-[isopropoxycarbonyloxy]ethyl ester, etc.); lower alkanesulfonyl(lower)alkyl ester (e.g., mesylmethyl ester, 2-mesylethyl ester, etc.); lower alkoxycarbonyloxy(lower)alkyl ester (e.g., methoxycarbonyloxymethyl ester, ethoxycarbonyloxymethyl ester, propoxycarbonyloxymethyl ester, t-butoxycarbonyloxymethyl ester, 1-(or 2-)-methoxycarbonyloxyethyl ester, 1-(or 2)-ethoxycarbonyloxyethyl ester, 1-(or 2-)-isopropoxycarbonyloxyethyl ester, etc.); phthalidylidene(lower)alkyl ester; (5-lower alkyl-2-oxo-1,3-dioxol-4-yl)(lower)alkyl ester [e.g., (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl ester, (5-ethyl-2-oxo-1,3-dioxol-4-yl)methyl ester, (5-propyl-2-oxo-1,3-dioxol-4-yl)ethyl ester, etc.]; mono(or di or tri)aryl(lower)alkyl ester, for example, mono(or di or tri)phenyl(lower)alkyl ester which may have one or more suitable substituent(s) (e.g., benzyl ester, 4-methoxybenzyl ester, 4-nitrobenzyl ester, phenethyl ester, trityl ester, benzhydryl ester, bis(methoxyphenyl)methyl ester, 3,4-dimethoxybenzyl ester, 4-hydroxy-3,4-di-t-butylbenzyl ester, etc.);

aryl ester which may have one or more suitable substituent(s) such as substituted or unsubstituted phenyl ester (e.g., phenyl ester, tolyl ester, t-butylphenyl ester, xylyl ester, mesityl ester, cumenyl ester, 4-chlorophenyl ester, 4-methoxyphenyl ester, etc.); tri(lower)alkyl silyl ester; lower alkylthioester (e.g., methylthioester, ethylthioester, etc.) and the like.

Suitable "protected amino" may include acylamino or an amino group substituted by a conventional protecting group such as mono(or di or tri)aryl(lower)alkyl, for example, mono(or di or tri)phenyl(lower)alkyl (e.g., benzyl, trityl, etc.) or the like.

Suitable "acyl" and "acyl moiety" in the terms "acylamino" and "acyloxy" may include carbamoyl, aliphatic acyl group and acyl group containing an aromatic ring, which is referred to as aromatic acyl, or heterocyclic ring, which is referred to as heterocyclic acyl.

Suitable example of said acyl may be illustrated as follows :

Carbamoyl; Thiocarbamoyl;

Aliphatic acyl such as lower or higher alkanoyl (e.g., formyl, acetyl, propanoyl, butanoyl, 2-methylpropanoyl, pentanoyl, 2,2-dimethylpropanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl, undecanoyl, dodecanoyl, tridecanoyl, tetradecanoyl, pentadecanoyl, hexadecanoyl, heptadecanoyl, octadecanoyl, nonadecanoyl, icosanoyl, etc.);

lower or higher alkoxycarbonyl (e.g., methoxycarbonyl, ethoxycarbonyl, t-butoxycarbonyl, t-pentyloxycarbonyl, heptyloxycarbonyl, etc.);

lower or higher alkylsulfonyl (e.g., methylsulfonyl, ethylsulfonyl, etc.);

lower or higher alkoxysulfonyl (e.g., methoxysulfonyl, ethoxysulfonyl, etc.);

cyclo(lower)alkylcarbonyl (e.g., cyclopentylcarbonyl,

cyclohexylcarbonyl, etc.); or the like;

Aromatic acyl such as

aroyl (e.g., benzoyl, toluoyl, naphthoyl, etc.);

ar(lower)alkanoyl [e.g., phenyl(lower)alkanoyl (e.g., phenylacetyl, phenylpropanoyl, phenylbutanoyl, phenylisobutanoyl, phenylpentanoyl, phenylhexanoyl, etc.), naphthyl(lower)alkanoyl (e.g., naphthylacetyl, naphthylpropanoyl, naphthylbutanoyl, etc.), etc.];

ar(lower)alkenoyl [e.g., phenyl(lower)alkenoyl (e.g., phenylpropenoyl, phenylbutenoyl, phenylmethacryloyl, phenylpentenoyl, phenylhexenoyl, etc.), naphthyl(lower)alkenoyl (e.g., naphthylpropenoyl, naphthylbutenoyl, etc.), etc.];

ar(lower)alkoxycarbonyl [e.g., phenyl(lower)alkoxycarbonyl (e.g., benzyloxycarbonyl, etc.), etc.];

aryloxycarbonyl (e.g., phenoxycarbonyl, naphthyloxycarbonyl, etc.);

aryloxy(lower)alkanoyl (e.g., phenoxyacetyl, phenoxypropionyl, etc.);

arylglyoxyloyl (e.g., phenylglyoxyloyl, naphthylglyoxyloyl, etc.);

arylcarbamoyl (e.g., phenylcarbamoyl, naphthylcarbamoyl, etc.);

arylsulfonyl (e.g., phenylsulfonyl, p-tolylsulfonyl, etc.); or the like;

Heterocyclic acyl such as

heterocycliccarbonyl;

heterocyclic(lower)alkanoyl (e.g., heterocyclicacetyl, heterocyclicpropanoyl, heterocyclicbutanoyl, heterocyclicpentanoyl, heterocyclichexanoyl, etc.);

heterocyclic(lower)alkenoyl (e.g., heterocyclicpropenoyl, heterocyclicbutenoyl, heterocyclicpentenoyl, heterocyclichexenoyl, etc.); heterocyclicglyoxyloyl; or the like; in which suitable "heterocyclic moiety" in the terms "heterocycliccarbonyl",

"heterocyclic(lower)alkanoyl", heterocyclic(lower)alkenoyl" and "heterocyclicglyoxyloyl" as mentioned above means, in more detail, saturated or unsaturated, monocyclic or polycyclic heterocyclic group containing at least one hetero-atom such as an oxygen, sulfur, nitrogen atom and the like.

And, especially preferable heterocyclic group may be heterocyclic group such as

unsaturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 to 4 nitrogen atom(s), for example, pyrrolyl, pyrrolinyl, imidazolyl, pyrazolyl, pyridyl, dihydropyridyl, pyrimidinyl, pyrazinyl, pyridazinyl, triazolyl (e.g., 4H-1,2,4-triazolyl, 1H-1,2,3-triazolyl, 2H-1,2,3-triazolyl, etc.), tetrazolyl (e.g., 1H-tetrazolyl, 2H-tetrazolyl, etc.), etc.;

saturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 to 4 nitrogen atom(s), for example, pyrrolidinyl, imidazolidinyl, piperidyl, piperazinyl, etc.;

unsaturated condensed heterocyclic group containing 1 to 4 nitrogen atom(s), for example, indolyl, isoindolyl, indolinyl, indolizinyl, benzimidazolyl, quinolyl, isoquinolyl, indazolyl, benzotriazolyl, etc.;

unsaturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, oxazolyl, isoxazolyl, oxadiazolyl (e.g., 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, 1,2,5-oxadiazolyl, etc.), etc.;

saturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, morpholinyl, sydnonyl, etc.;

unsaturated condensed heterocyclic group containing 1

to 2 oxygen atom(s) and 1 to 3 nitrogen atom(s), for example, benzoxazolyl, benzoxadiazolyl, etc.;

unsaturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s), for example, thiazolyl, isothiazolyl, thiadiazolyl (e.g., 1,2,3-thiadiazolyl, 1,2,4-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, etc.), dihydrothiazinyl, etc.;

saturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s), for example, thiazolidinyl, etc.;

unsaturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing 1 to 2 sulfur atom(s), for example, thienyl, dihydrodithiinyl, dihydrodithionyl, etc.;

unsaturated condensed heterocyclic group containing 1 to 2 sulfur atom(s) and 1 to 3 nitrogen atom(s), for example, benzothiazolyl, benzothiadiazolyl, etc.;

unsaturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing an oxygen atom, for example, furyl, etc.;

unsaturated 3 to 8-membered (more preferably 5 or 6-membered) heteromonocyclic group containing an oxygen atom and 1 to 2 sulfur atom(s), for example, dihydrooxathiinyl, etc.;

unsaturated condensed heterocyclic group containing 1 to 2 sulfur atom(s), for example, benzothienyl, benzodithiinyl, etc.;

unsaturated condensed heterocyclic group containing an oxygen atom and 1 to 2 sulfur atom(s), for example, benzoxathiinyl, etc.; and the like.

The acyl moiety as stated above may have one to ten, same or different, suitable substituent(s) such as lower alkyl as exemplified above, lower alkoxy as exemplified

above, lower alkylthio wherein lower alkyl moiety is as exemplified above, lower alkylamino wherein lower alkyl moiety is as exemplified above, cyclo(lower)alkyl, cyclo(lower)alkenyl, halogen as exemplified above, aryl as exemplified above, amino, protected amino as exemplified above, hydroxy, protected hydroxy, cyano, nitro, carboxy, protected carboxy as exemplified above, sulfo, sulfamoyl, imino, oxo, amino(lower)alkyl wherein lower alkyl moiety is as exemplified above, carbamoyloxy, mono(or di or tri)halo(lower)alkyl wherein halogen moiety and lower alkyl moiety are each as exemplified above, hydroxy(lower)alkyl wherein lower alkyl moiety is as exemplified above, or the like.

The processes for preparing the object and starting compounds are explained in detail in the following.

Process (1)

The compound (I) or a salt thereof can be prepared by reacting the compound (II) or a salt thereof with the compound (III) or a salt thereof.

This reaction is usually carried out in a solvent such as water, alcohol (e.g., methanol, ethanol, etc.), benzene, N,N-dimethylformamide, tetrahydrofuran, toluene, methylene chloride, ethylene dichloride, chloroform, diethyl ether or any other solvent which does not adversely affect the reaction.

The reaction temperature is not critical and the reaction is usually carried out under warming to heating.

Process (A) - (1)

The compound (VI) or a salt thereof can be prepared by reacting the compound (IV) or a salt thereof with the compound (V) or a salt thereof.

This reaction is usually carried out in a solvent

such as water, alcohol (e.g., methanol, ethanol, etc.), benzene, N,N-dimethylformamide, tetrahydrofuran, toluene, methylene chloride, ethylene dichloride, chloroform, diethyl ether or any other solvent which does not adversely affect the reaction.

The reaction temperature is not critical and the reaction is usually carried out under warming to heating.

When the starting compound is in liquid, it can be also used as a solvent.

Process (A) - (2)

The compound (II) or a salt thereof can be prepared by subjecting the compound (VI) or a salt thereof to reduction reaction.

Reduction is carried out in a conventional manner, including chemical reduction and catalytic reduction.

Suitable reducing reagent to be used in chemical reduction are hydrides (e.g., hydrogen iodide, hydrogen sulfide, lithium aluminum hydride, sodium borohydride, sodium cyanoborohydride, etc.) or a combination of a metal (e.g., tin, zinc, iron, etc.) or metallic compound (e.g., chromium chloride, chromium acetate, etc.) and an organic acid or an inorganic acid (e.g., formic acid, acetic acid, propionic acid, trifluoroacetic acid, p-toluenesulfonic acid, hydrochloric acid, hydrobromic acid, etc.).

Suitable catalysts to be used in catalytic reduction are conventional ones such as platinum catalysts (e.g., platinum plate, spongy platinum, platinum black, colloidal platinum, platinum oxide, platinum wire, etc.), palladium catalysts (e.g., spongy palladium, palladium black, palladium oxide, palladium on carbon, colloidal palladium, palladium on barium sulfate, palladium on barium carbonate, etc.), nickel catalysts (e.g., reduced nickel, nickel oxide, Raney nickel, etc.), cobalt catalysts (e.g., reduced cobalt, Raney cobalt, etc.), iron catalysts (e.g.,

reduced iron, Raney iron, etc.), copper catalysts (e.g., reduced copper, Raney copper, Ullman copper, etc.) and the like.

The reduction is usually carried out in a conventional solvent which does not adversely influence the reaction such as water, alcohol (e.g., methanol, ethanol, propanol, etc.), tetrahydrofuran, dioxane, N,N-dimethylformamide, etc., or a mixture thereof.

The reaction temperature is not critical and the reaction is usually carried out under cooling to warming.

Process (B)

The compound (VII) or a salt thereof can be prepared by subjecting the compound (VIa) or a salt thereof to elimination reaction of the amino protective group.

The reaction can be carried out in the manner disclosed in Preparation 3 or similar manners thereto.

Process (C)

The compound (VIb) or a salt thereof can be prepared by subjecting the compound (VII) or a salt thereof to acylation reaction.

The reaction can be carried out in the manner disclosed in Preparation 4 or similar manners thereto.

Suitable salts of the object and the starting compounds in Processes (1) and (A)-(C) can be referred to the ones as exemplified for the compound (I).

The new pyridopyrazine derivatives (I) and pharmaceutically acceptable salts thereof hardly possess a strong inhibitory activity against phosphodiesterase III (PDE III), but possess a strong inhibitory activity against phosphodiesterase IV (PDE IV) and a strong inhibitory activity on the tumor necrosis factor (TNF).

That is, the pyridopyrazine derivatives (I) and pharmaceutically acceptable salts thereof are selective

inhibitors of phosphodiesterase IV (PDE IV) and inhibitors on the production of tumor necrosis factor (TNF).

Accordingly, the new pyridopyrazine derivatives (I) and a pharmaceutically acceptable salt thereof can be used for prophylactic and therapeutic treatment of PDE-IV and TNF mediated diseases such as chronic inflammatory diseases (e.g., rheumatoid arthritis, osteoarthritis, emphysema, chronic bronchiolitis, etc.), osteoporosis, rejection by transplantation, asthma, eosinophilia, cystic fibrosis, hepatitis, pancreatitis, nephritis, endotoxin shock, specific autoimmune diseases [e.g., ankylosing spondylitis, autoimmune hematological disorders (e.g., hemolyticodo anaemia, aplastic anaemia, pure red cell anaemia, idiopathic thrombocytopenia, etc.), systemic lupus erythematosus, polychondritis, scleroderma, Wegener granulamotosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, atopic dermatitis, psoriasis, idiopathic sprue, autoimmune inflammatory bowel disease (e.g., ulcerative colitis, Crohn's disease, etc.), endocrine ophthalmopathy, Grave's disease, sarcoidosis, multiple sclerosis, primary biliary cirrhosis, juvenile diabetes (diabetes mellitus type I), Reiter's syndrome, non infection uveitis, autoimmune keratitis (e.g., keratoconjunctivitis sicca, vernal keratoconjunctivitis, etc.), interstitial lung fibrosis, psoriatic arthritis, etc.], cancer cachexia, AIDS cachexia, thrombosis, and the like.

In order to show the utilities of the pyridopyrazine derivatives (I) and a pharmaceutically acceptable salt thereof of the present invention, pharmacological test data of the representative compound of the pyridopyrazine derivatives (I) are illustrated in the following.

(a) Inhibition of U937 phosphodiesterase IV (PDE IV)

1. Test method :

Harvested U937 was freezed in -80°C and throwed to destroy the cell body. The pellet of destroyed cell was washed by Phosphate-buffered saline (PBS).

The washed cell pellet was homogenized with Dounce homogenizer (20 strokes) in homogenizing buffer (0.5% deoxycholate [DOC], 5 mM 2-mercaptoethanol, 1 µM leupeptin, 100 µM PMSF, 20 µM p-tosyl-L-lysine-chloromethyl ketone (TLCK] in PBS). The homogenate was centrifuged at 100,000 g x 90 minutes (4°C) and the supernatant containing PDE IV activity was dialyzed against dialysis buffer, which was the same component as homogenizing buffer without DOC. The dialyzed supernatant of homogenate was stored in freezer (-80°C) as PDE IV enzyme preparation.

Enzyme preparation was diluted in assay buffer (10 mM Tris-HCl, 5 mM MgCl, 1 mM 2-Mercaptoethanol [pH 8.0]). In advance the rate of dilution was choosen every new lot of homogenizing preparation. For blank, a part of the enzyme preparation was boiled for 10 minutes.

Test compounds were dissolved in dimethylsulfoxide (DMSO) at a concentration of 4 x 10(-2)[M] (final conc. 1 x 10(-5)M), then serial dilutions were made in DMSO to achieve desired concentrations. The diluted compounds of each concentration were further diluted 1:500 in assay buffer (0.2% DMSO). Final DMSO concentration in assay tube was 0.025%.

In duplicate, the followings were added to a glass tube, in order, at 0°C (all concentrations are given as final concentrations in assay tube).

50 µl compound of assay buffer for control or blank

50 µl 8 x 10(-5)[M] CI-930 (final 10 µM) : (CI-930 is PDE III inhibitor)

200 µl enzyme preparation or boiled enzyme

preparation for blank.

The reaction tube was preincubated in a water bath (30°C) for 5 minutes, then 100 µl [^{3}H]-cAMP (37.0 MBq/ml [^{3}H]-cAMP : 4 µM cold cAMP = 1:800) was added thereto. After 15 minutes, 2.5 units/ml alkaline phosphatase was added to the reaction mixture and the reaction was continued for 15 minutes. Dowex 1 x 8 gel was added to the reaction mixture and was vortexed well. The mixture was centrifuged at 1000 rpm x 5 minutes, and then 500 µl of the supernatant was added to 10 ml scintillation fluid in appropriate vial, vortexed, and counted for [^{3}H].

The inhibitory activity was calculated according to the following equation :

$$\% \text{ Inhibition} = 100 - \frac{\text{avg.cpm[test compound]} - \text{avg.cpm[blank(boiled enzyme)]}}{\text{avg.cpm[control(no compound)]} - \text{avg.cpm[blank(boiled enzyme)]}} \times 100$$

2. Test compound :

(a) 2-Isobutyl-3-oxo-4-[3-[3-(2-methoxyphenyl)ureido]-phenyl]-3,4-dihydropyrido[2,3-b]pyrazine

3. Test result :

Test compound	IC_{50} (M)
(a)	2.4×10^{-8}

(b) Inhibition on TNF-α production in human mononuclear cells

1. Test method :

Blood was drawn from healthy volunteers with heparin. The mononuclear cell (MNC) fraction was obtained by gradient centrifugation (1800 rpm, 15 minutes), diluted with the same volume of RPMI-1640 culture medium, over Ficoll-Paque (Pharmacia LKB Biotechnology). MNC were washed twice with RPMI-1640. Then, MNC were resuspended in RPMI-1640 culture medium supplemented with 2 mM L-glutanine and 1% fetal bovine serum. MNC were incubated at 37°C for 16 hours in 96-well micro culture plate at a concentration of 3×10^{-5} cells/well with or without 1 µg/ml lipopoly saccharide (LPS) (from E. coli) and various amounts of test compound. At the end of incubation, the supernatant was obtained and its TNF-α activity was measured by enzyme-linked immunosorbent assay (ELISA). ELISA was performed with TNF-α ELISA kit (Otsuka Pharmaceutical Co., Ltd.).

2. Test compound :

(a) 2-Isobutyl-3-oxo-4-[3-[3-(2-methoxyphenyl)ureido]-phenyl]-3,4-dihydropyrido[2,3-b]pyrazine

3. Test result :

Test compound	IC_{50} (M)
(a)	3.1×10^{-8}

For therapeutic administration, the object compounds (I) of the present invention and pharmaceutically acceptable salts thereof are used in a form of the conventional pharmaceutical preparation in admixture with a conventional pharmaceutically acceptable carrier such as an organic or inorganic solid or liquid excipient which is

suitable for oral, parenteral or external administration. The pharmaceutical preparation may be compounded in a solid form such as granule, capsule, tablet, dragee or suppository, or in a liquid form such as solution, suspension or emulsion for injection, ingestion, eye drops, etc. If needed, there may be included in the above preparation auxiliary substance such as stabilizing agent, wetting or emulsifying agent, buffer or any other commonly used additives.

The effective ingredient may usually be administered with a uint dose of 0.001 mg/kg to 500 mg/kg, preferably 0.01 mg/kg to 10 mg/kg, 1 to 4 times a day. However, the above dosage may be increased or decreased according to age, weight and conditions of the patient or the administering method.

Preferred embodiments of the object compound (I) are as follows.

R^1 is lower alkyl, carboxy or esterified carboxy (more preferably lower alkoxycarbonyl), and

R^2 is phenyl which may have acylamino [more preferably lower alkanoylamino, or arylcarbamoyl (more preferably phenylcarbamoyl) which may have 1 to 3 (more preferably one or two; most preferably one) suitable substituent(s) (more preferably lower alkoxy)].

More preferred embodiments of the object compound (I) are as follows.

R^1 is lower alkyl, carboxy or lower alkoxycarbonyl, and

R^2 is phenyl, lower alkanoylaminophenyl or (lower alkoxyphenyl)carbamoylaminophenyl.

The following Preparations and Examples are given for

the purpose of illustrating the present invention in more detail.

Preparation 1

A mixture of 2-(3-acetamidophenyl)amino-3-nitropyridine (5.85 g) and 10% palladium on carbon (0.8 g) in ethanol (100 ml) and 1,4-dioxane (100 ml) was stirred under hydrogen (3 atm) at room temperature for 3 hours. The catalyst was removed and the solvent was evaporated. The resultant solids were collected and washed with isopropyl ether to give 2-(3-acetamidophenyl)amino-3-aminopyridine (5.05 g).

NMR (DMSO-d_6, δ) : 2.03 (3H, s), 5.09 (2H, s), 6.63 (1H, dd, J=5Hz, 8Hz), 6.89 (1H, dd, J=1.5Hz, 8Hz), 7.0-7.25 (2H, m), 7.33 (1H, m), 7.49 (1H, dd, J=1.5Hz, 5Hz), 7.71 (1H, s), 7.87 (1H, s), 9.80 (1H, s)

Preparation 2

A mixture of 2-chloro-3-nitropyridine (6.12 g), 3'-aminoacetanilide (5.80 g) and potassium carbonate (5.34 g) in toluene (50 ml) was refluxed for 5 hours. The mixture was cooled and the solids were collected, washed with water, ethanol and isopropyl ether successively to give 2-(3-acetamidophenylamino)-3-nitropyridine (5.88 g) as an orange solid.

NMR (DMSO-d_6, δ) : 2.06 (3H, s), 6.99 (1H, dd, J=5Hz, 8Hz), 7.2-7.4 (3H, m), 7.91 (1H, s), 8.5-8.6 (2H, m), 9.93 (1H, s), 9.99 (1H, s)

Preparation 3

A mixture of 2-(3-acetamidophenylamino)-3-nitropyridine (10 g) and 3N hydrochloric acid (100 ml) was stirred under reflux for an hour. After cooling, the reaction was adjusted to pH 7 with sodium bicarbonate

solution, and precipitated materials were collected, washed with water and dried to give 2-(3-aminophenylamino)-3-nitropyridine (8.2 g).

NMR ($CDCl_3$, δ) : 3.75 (2H, br s), 6.51 (1H, m), 6.81 (1H, m), 6.96 (1H, m), 7.15 (2H, m), 8.50 (2H, m), 10.05 (1H, br s)

Preparation 4

A mixture of 2-(3-aminophenylamino)-3-nitropyridine (0.65 g) and 2-methoxyphenyl isocyanate (0.63 g) in dioxane (10 ml) was refluxed for 2 hours. After evaporation of the solvent, crude crystals were washed with ethanol to give 3-nitro-2-[3-[3-(2-methoxyphenyl)ureido]phenylamino]pyridine (1.02 g).

NMR (DMSO-d_6, δ) : 3.89 (3H, s), 7.00 (4H, m), 7.27 (3H, m), 7.81 (1H, m), 8.12 (1H, m), 8.26 (1H, s), 8.55 (2H, m), 9.39 (1H, s), 9.93 (1H, s)

Preparation 5

A solution of 3-nitro-2-[3-[3-(2-methoxyphenyl)ureido]phenylamino]pyridine (11.65 g) in dioxane (200 ml) and methanol (100 ml) containing 10% palladium on charcoal (0.8 g) was hydrogenated at 3 atoms at room temperature. After removal of the catalyst and evaporation of solvents, residual crystals were washed with ether and dried to give 3-amino-2-[3-[3-(2-methoxyphenyl)uredio]phenylamino]pyridine (12.31 g).

NMR (DMSO-d_6, δ) : 3.90 (3H, s), 5.10 (2H, s), 6.63 (1H, m), 6.90 (3H, m), 7.03 (2H, m), 7.13 (1H, m), 7.25 (1H, m), 7.50 (1H, m), 7.71 (1H, m), 7.77 (1H, m), 8.15 (1H, m), 8.24 (1H, s), 9.20 (1H, s)

Example 1

A mixture of 2-phenylamino-3-aminopyridine (0.5 g)

and 2-oxobutyric acid (276 mg) in ethanol (10 ml) was refluxed for 2 hours. The reaction mixture was cooled and the precipitate was filtrated by suction. The crystal was washed with ethanol to obtain 2-ethyl-3-oxo-4-phenyl-3,4-dihydropyrido[2,3-b]pyrazine (594 mg).

NMR (DMSO-d_6, δ) : 1.28 (3H, t, J=7Hz), 2.89 (2H, q, J=7Hz), 7.27-7.61 (6H, m), 8.25 (1H, d, J=10Hz), 8.37 (1H, d, J=5Hz)

Example 2

The following compounds were obtained according to a similar manner to that of Example 1.

(1) 2-Methyl-3-oxo-4-phenyl-3,4-dihydropyrido[2,3-b]-pyrazine
Mass (FAB) m/e : 238 (M+1)

(2) 2-Hexyl-3-oxo-4-phenyl-3,4-dihydropyrido[2,3-b]-pyrazine
NMR (DMSO-d_6, δ) : 0.88 (3H, t, J=7Hz), 1.25-1.50 (6H, m), 1.75 (2H, quint, J=6Hz), 2.87 (2H, t, J=8Hz), 7.3-7.52 (6H, m), 8.25 (1H, dd, J=1Hz, 10Hz), 8.37 (1H, dd, J=1Hz, 5Hz)

(3) 2-Hexyl-3-oxo-4-[3-[3-(2-methoxyphenyl)-ureido]phenyl]-3,4-dihydropyrido[2,3-b]pyrazine
NMR (DMSO-d_6, δ) : 0.90 (3H, m), 1.2-1.5 (6H, m), 1.75 (2H, m), 2.85 (2H, m), 4.90 (3H, s), 6.9-7.1 (4H, m), 7.4 (3H, m), 7.58 (1H, s), 8.1 (1H, m), 8.25 (2H, m), 8.4 (1H, m), 9.50 (1H, s)

(4) 2-Propyl-3-oxo-4-[3-[3-(2-methoxyphenyl)-ureido]phenyl]-3,4-dihydropyrido[2,3-b]pyrazine
mp : 198-200°C
NMR (DMSO-d_6, δ) : 1.02 (3H, t, J=7Hz), 1.80 (2H,

m), 2.85 (2H, t, J=7Hz), 3.88 (3H, s), 6.85-7.1 (4H, m), 7.42 (3H, m), 7.57 (1H, s), 8.08 (1H, m), 8.26 (2H, m), 8.40 (1H, m), 9.52 (1H, s)

(5) 2-[(1RS)-1-Methylpropyl]-3-oxo-4-[3-[3-(2-methoxyphenyl)ureido]phenyl]-3,4-dihydropyrido[2,3-b]pyrazine
mp : 214-215°C
NMR (DMSO-d_6, δ) : 0.95 (3H, t, J=7Hz), 1.27 (3H, d, J=7Hz), 1.57 (1H, m), 1.87 (1H, m), 3.35 (1H, m), 3.89 (3H, s), 6.8-7.1 (4H, m), 7.45 (3H, m), 7.58 (1H, s), 8.09 (1H, d, J=7Hz), 8.28 (2H, m), 8.40 (1H, m), 9.52 (1H, s)

(6) 2-Isobutyl-3-oxo-4-[3-[3-(2-methoxyphenyl)ureido]-phenyl]-3,4-dihydropyrido[2,3-b]pyrazine
mp : 211-213°C
NMR (DMSO-d_6, δ) : 1.00 (6H, d, J=7Hz), 2.30 (1H, m), 2.75 (2H, d, J=7Hz), 3.88 (3H, s), 6.8-7.05 (4H, m), 7.40 (3H, m), 7.60 (1H, s), 8.10 (1H, d, J=7Hz), 8.27 (2H, m), 8.40 (1H, m), 9.52 (1H, s)

(7) 2-Ethoxycarbonyl-3-oxo-4-[3-[3-(2-methoxyphenyl)-ureido]phenyl]-3,4-dihydropyrido[2,3-b]pyrazine
mp : 177-178°C
NMR (DMSO-d_6, δ) : 1.33 (3H, t, J=7Hz), 3.89 (3H, s), 4.40 (2H, q, J=7Hz), 6.8-7.05 (4H, m), 7.45 (3H, m), 7.65 (1H, s), 8.10 (1H, d, J=7Hz), 8.30 (1H, s), 8.39 (1H, d, J=7Hz), 8.53 (1H, d, J=3Hz)

(8) 2-Ethoxycarbonyl-3-oxo-4-(3-acetamidophenyl)-3,4-dihydropyrido[2,3-b]pyrazine
mp : 182-185°C

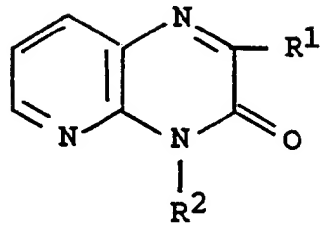
NMR (DMSO-d_6, δ) : 1.34 (3H, t, J=7Hz), 2.06 (3H, s), 4.41 (2H, q, J=7Hz), 7.05 (1H, m), 7.50 (2H, m), 7.60 (1H, m), 7.70 (1H, m), 8.38 (1H, d, J=7Hz), 8.52 (1H, m), 10.15 (1H, s)

(9) 2-Ethyl-3-oxo-4-[3-[3-(2-methoxyphenyl)ureido]-phenyl]-3,4-dihydropyrido[2,3-b]pyrazine
NMR (DMSO-d_6, δ) : 1.30 (3H, t, J=7Hz), 2.90 (2H, q, J=7Hz), 3.86 (3H, s), 6.85-7.15 (4H, m), 7.40 (3H, m), 7.58 (1H, s), 8.05-8.5 (4H, m), 9.50 (1H, s)

(10) 4-(3-Acetamidophenyl)-2-ethyl-3-oxo-3,4-dihydro-pyrido[2,3-b]pyrazine
NMR (DMSO-d_6, δ) : 1.28 (3H, t, J=7Hz), 2.05 (3H, s), 2.88 (2H, q, J=7Hz), 6.99 (1H, m), 7.40 (1H, m), 7.46 (1H, d, J=7Hz), 7.58 (1H, m), 7.64 (1H, s), 8.25 (1H, d, J=7Hz), 8.37 (1H, d, J=3Hz)

What we claim is :

1. A compound of the formula :

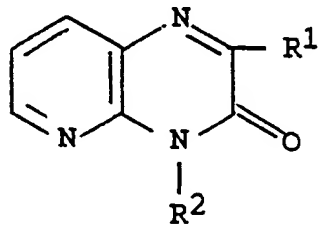


wherein R^1 is lower alkyl, carboxy or protected carboxy, and

R^2 is aryl which may have protected amino,

and a pharmaceutically acceptable salt thereof.

2. A process for preparing a compound of the formula :

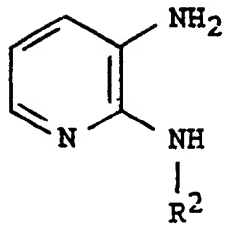


wherein R^1 is lower alkyl, carboxy or protected carboxy, and

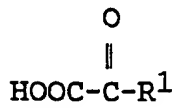
R^2 is aryl which may have protected amino,

or a salt thereof,

by reacting a compound of the formula :



wherein R^2 is as defined above,
or a salt thereof with a compound of the formula :



wherein R^1 is as defined above,
or a salt thereof.

The Patent Office

28

Application No: GB 9414453.2 **Examiner:** Diane Davies
Claims searched: 1-2 **Date of search:** 4 October 1995

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.N):

Int Cl (Ed.6): C07D 471/04

Other: Online databases: CAS-ONLINE, EDOC, JAPIO, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X	EP 0008864 A	(Fisons Ltd.) Whole document: antiinflammatory 4-phenyl-pyrido[2,3-b]pyrazin-3-ones.	1-2

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.